Production: **Cell/Tissue Lysis Buffer**
Catalog No:RC0005

Product Information

Obtaining intact protein samples from cells or tissues is one of the critical factors influencing the authenticity and reliability of Western blotting (WB) results. This product is a revolutionary solution specifically designed to extract complete protein samples, overcoming the limitations of traditional RIPA lysis buffers and ultrasonic disruption techniques. It enables rapid and complete extraction of proteins from cells and tissues while effectively protecting protein chemical modifications. It is particularly suitable for the extraction of large molecular weight proteins and post-translationally modified proteins, ensuring high-quality samples for protein analysis experiments such as Western blotting.

Components

Components	Size (20mL)	Size (50mL)	Size (100mL)
Reagent A	40 μL	100 μL	200 μL
Reagent B	20 mL	50 mL	100 mL

Key Features

- Complete extraction of large molecular weight proteins.
- Protection of post-translational modifications (e.g., phosphorylation, glycosylation, ubiquitination, methylation, and acetylation).
- One-step process without requiring additional enzyme inhibitors.
- Compatible with both cell and tissue samples.

Storage

Store Reagent A at -20°C. Store Reagent B at room temperature or 4°C
Note: Precipitation may occur when Reagent B is stored at 4 °C over a long time, but it does not affect product quality. The precipitation will redissolve at room temperature.

Application

Extraction of large macromolecular protein.

Extraction of post-translationally modified proteins (e.g., glycosylated, phosphorylated, ubiquitinated).

Western blot analysis.

Operation steps

For Adherent cell

1. Adding 2 μL of Reagent A into 1 mL of Reagent B immediately before use(Ratio of Reagent A and B:

500:1). Mix thoroughly by vortexing and place on ice
Note: Calculate the volume of the lysis buffer you need in advance according to Step 3; discard the unused buffer after use.
2. Discard the cell culture medium and wash the cells twice with ice-cold PBS.
3. Place the culture dish/plate on ice or ice water and add 1 mL of the premixed lysis buffer per 5x10^6 cells (e.g. add 200 µL of lysis buffer to a 35 mm dish containing 1x10^6 cells). Keep the dish/plate on ice for an additional 5 min and swirl occasionally to allow the lysis buffer to fully cover the cells.
4. After 5 min of lysis, scrape the cells off the dish/plate by a clean plastic scraper and collect the lysate into a centrifuge tube.
5. Vortex the lysates thoroughly (3 x 10 sec) and place the lysates on ice or ice water for another 10 min to complete the lysis.
6. Heat the lysates on a 95°C heat block for 5 min.
7. Cool the lysates on ice or ice water for 3 min.
8. Centrifuge the lysates at 13,000g for 5 min at 4°C.
9. Measure the protein concentration using a spectrophotometer or SDS compatible protein assay.
10. If for further use, store the lysates at -20°C.
Note: For reducing SDS-PAGE, a final concentration of 2–5% β-mercaptoethanol or 50 mM DTT, plus 0.1% bromophenol blue, must be added to the lysates. Samples should be heated at 95°C for 5 min before loading

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For Suspension cell

1. Prepare Cell/Tissue Lysis buffer immediately before use according to Step 1 in the adherent cell protocol.
2. Centrifuged the suspended cells at 300 g for 5 min, discard the supernatant, and resuspend the cells with 10 mL of ice-cold PBS. Centrifuge again, discard the PBS, and resuspend the cells into the residual PBS with a pipette.
3. Add 1mL of premixed Cell/Tissue Lysis buffer to per 5x10^6 cells, mix well through a pipette, and then place in ice or ice water for 15 min.
4. Follow steps 6-10 in the adherent cell protocol.

For Tissue protein

1. Prepare Cell/Tissue Lysis buffer immediately before use according to Step 1 in the adherent cell protocol.
2. In liquid nitrogen, grind tissue into fine particles using a mortar and pestle.
3. Add the frozen tissue powder into the premixed Cell/Tissue Lysis buffer at the ratio of 1g of tissue to 3 mL of lysis buffer.
4. Using a homogenizer to homogenize the tissue.
Note: keep the tubes on the ice when homogenizing to avoid homogenization heats the sample
5. Incubate homogenized samples on ice for at least 15 min for complete lysis.
Note: If there are multiple samples, keep all homogenized samples on ice until the last sample is done.
6. 15 min after homogenization of the last sample, centrifuge at 13,000 g at 4°C for 10 min. Transfer the supernatant with extracted proteins to a clean centrifuge tube.
7. Follow Steps 6-10 in the adherent cell protocol.